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ORIGINAL PAPER

Nitrogen retranslocation, allocation, and utilization in bare root *Larix olgensis* seedlings

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Abstract: We quantified biomass accumulation and nitrogen (N) retranslocation, allocation, and utilization of Changbai larch (*Larix olgensis*) seedlings subjected to four fertilization treatments (24, 59, 81, 117 $kg \cdot ha^{-1}$ N) with an unfertilized control during summer and autumn 2009. Ammonium phosphate (18-46-0) was the fertilizer used in all treatments. On both sampling dates, the needles had greater biomass and N content than new (2009) stems and old (2008) stems, and coarse, medium and fine roots (diameters of >5, 2–5 mm, and 0–2 mm, respectively). Higher N concentration was observed in old stems and coarse roots than that in new stems and medium roots. In mid-summer, fine roots had higher N concentration than coarse roots. The treatment with 24 $kg \cdot ha^{-1}$ N had the greatest biomass and N content in needles and old stems, and highest net N retranslocation (*NRA*) and amount of N derived from soil. On September 21, no N translocation was observed, while the treatment with 24 $kg \cdot ha^{-1}$ N had the highest N utilization efficiency and fertilizer efficiency. Vector analysis revealed that all four fertilization treatments induced N excess relative to the control. The treatments with 59, 81, 117 $kg \cdot ha^{-1}$ N induce N excess compared with treatments at 24 $kg \cdot ha^{-1}$ N. We conclude that the traditional local fertilizer application rates exceeded N requirements and N uptake ability for Changbai larch seedlings. The application rate of 24 $kg \cdot ha^{-1}$ N is recommended.

Keywords: *Larix olgensis*; Nitrogen; retranslocation; allocation; biomass; Vector analysis

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Introduction

Nitrogen (N) availability is one of the important factors influencing growth of tree seedlings (Salifu and Timmer 2001, 2003; Salifu et al. 2008, 2009; Bown et al. 2010). Storage of N is important for young trees because buds break in the spring is sub-optimal when N uptake by the roots before photosynthesis can provide carbon skeletons for amino acid synthesis (Millard and Neilsen 1989). Deciduous trees store N mainly in woody stems and roots, while evergreen trees store N mainly in shoots (Millard and Grelet 2010).

Retranslocation of stored nutrient is important for the production of new tissues at all stages of development from the seedling to the mature tree (Nambiar and Fife 1991). Studies have focused on nutrient retranslocation in juvenile evergreen conifer seedlings (Nambiar and Fife 1987, 1991; van den Driessche 1984; Imo and Timmer 2001; Salifu and Timmer 2001, 2003), hardwood seedlings (Salifu et al. 2008, 2009), and mature tree stands (Helmisaari 1992; Mead and Preston 1994; Chapin and Kedrowski 1983). The main conclusions from these reports include: (1) the leaf is the main source and sink of retranslocated nutrients in evergreen conifers (Nambiar and Fife 1987, 1991). (2) net retranslocation is mainly driven by the amount of plant N reserves established before planting (Nambiar and Fife 1987; Imo and Timmer 2001; Salifu and Timmer 2001, 2003). (3) retranslocation is more efficient in trees growing on less fertile soils (Miller et al. 1979; Imo and Timmer 2001; Salifu and Timmer 2001). It is, however, not clear whether nutrient retrans-

location from nutrient reserves in the whole seedling, such as from combined stems and roots (CSR) in deciduous conifers, follows the same pattern. Most previous studies focused on N retranslocated from shoots only (Salifu and Timmer 2001).

In response to external resource changes, trees tend to allocate internal resources to the tissues responsible for acquiring the most limiting compounds (Welker et al. 1991; Lehtilä et al. 2000; Ueda et al. 2009). N allocation to shoots improves carbon acquisition for carbohydrate production, and photosynthetic capacity is strongly correlated with the N content of leaves (Evans 1989). The decrease in N concentration in green leaves may relate to an increase in the proportion of plant N invested in roots (Lutze and Gifford 1998). Mean N concentration was observed to be significantly higher in smaller roots (< 2 mm diameter) than in larger roots (2–5 mm) (Gordon and Jackson 2000). However, most research has focused on evergreen plants or plants in leaf. There has been little research on patterns of N allocation over seasons within deciduous woody stock.

Changbai larch (*Larix olgensis* H.) is an important commercial timber tree species in north-eastern China (Zhu et al. 2008). Prior to this study, the local fertilizer regime for growing Changbai larch seedlings appeared to result in sub-optimal seedling quality (unpublished data). Therefore, we investigated the growth and N translocation, allocation, and utilization of transplanted bare root Changbai larch seedlings to find an optimal fertilizer regime for this species. We hypothesized that Changbai larch seedlings would support growth of new tissues by N retranslocation from CSR rather than by N uptake from soils, and N would be allocated mostly to needles and fine roots.

Materials and methods

Seeds of Changbai larch were collected by employees of Xiaobeihu Forest Station (128°28' E, 44°03' N) from a local wild stand in Heilongjiang province, northeast China. After the collection, the seeds were transported to Jiangmifeng nursery (43°45' N, 126°45' E), Jilin City, Jilin Province, north-eastern China, and stored at 0–4°C. In Jilin, annual precipitation is 650–750 mm, with less than 200 mm from May to mid-June. The average annual temperature is 3–5°C, and the average temperature in the early growing season ranges from 4 to 9°C.

Seeds were soaked in 5% potassium permanganate (W/W) solution for 24 h and stratified for 5 d at 0–4°C. Then the seeds were sowed in a nursery bed at the density of 700 seed·m^{-2} in late April 2008. In mid June 2008, the germinated seedlings were thinned to about 550 seedling·m^{-2}. After excavation from the seedling bed in October 2008 and cold storage until mid April 2009, the seedlings were transplanted into another nursery bed with initial properties of surface soil (0–20cm) determined (Bao 2000): pH of 6.08, electrical conductivity (25°C) of 0.15 dS·m^{-1}, total N of 2.07 mg·g^{-1}, NH_4^+-N of 1.88 mg·kg^{-1}, NO_3^--N of 277.98 mg·kg^{-1} and organic C of 24.28 mg·g^{-1}.

Locally prescribed fertilizer applications in 2008 included: ammonium phosphate (18-46-0) incorporated into surface soil before sowing at 54 kg·ha^{-1} N in late April, and two top-dress applications of a 1:1 combination of granular ammonium nitrate (21-0-0) and urea (46-0-0) at 65 and 75 kg·ha^{-1} N in late June and early July, respectively. The experiment began in the spring of 2009. There were four fertilizer treatments (24, 59, 81, 117 kg·N·ha^{-1}) and an unfertilized control treatment. Fertilizer was applied as ammonium phosphate (18-46-0), incorporated into surface soils in mid April, 2009. A fertilizer rate of 81 kg·N·ha^{-1} is operationally employed for local seedling production. For each treatment, there were three randomly assigned plots (replicates). Each plot had an area of 1 m × 1 m with 0.1–0.2 m buffer between plots and plastic barriers inserted to a depth of 0.2 m within each buffer to eliminate lateral movement of fertilizer between plots. All plots shared the same irrigation regime performed by automatic sprayers installed along the seed beds. Weed control was conducted manually. Frequency and intensity of irrigation and weed control were adjusted based on seedling growth rates and weather conditions throughout the growing season.

On both July 2 and September 21, 2009, 150 seedlings (10 seedling·plot^{-1} × 3 plot·treatment^{-1} × 5 treatments) were randomly excavated from the nursery bed. All seedlings were divided into shoot and root fractions at the cotyledon scar. Shoots were divided into needles, old (2008) stems (dark brown), new (2009) stems (light brown). Roots were divided into coarse roots (>5 mm in diameter), medium roots (2–5 mm in diameter) and fine roots (0–2 mm in diameter). The color of coarse and medium roots was dark brown, and the color of most fine roots was white. For each tissue fraction, the ten samples per plot and treatment were bulked and then transported to the laboratory on ice (0–2°C), where roots were washed free of soil. Dry biomass was measured for each bulked tissue after oven-drying for 48 h at 70°C. Bulked tissue samples were ground to pass a 1 mm sieve, then one 0.2 g-sample for each tissue was digested in 5 mL of H_2O_2-H_2SO_4 and diluted to 50 mL. Total seedling N concentration was measured with 5mL of digestion solution using an automatic N analyzer (UDK 152 automatic N analyzer, VELP Co., Usmate (MB), Italy).

Net N retranslocation amount (*NRA*) was computed as (Salifu and Timmer 2001):

$$NRA = A - B \tag{1}$$

Where, A is the total N content (storage) of the whole seedling before transplanting in mid April 2009 (old stem + intact root). In this study, A=14.51 mg·seedling^{-1} N. B is the total N content in old tissues (old stem + dark brown root > 2 mm diameter) on July 2 or September 21. Following Salifu and Timmer (2001), the N content in dead tissue and N leached from seedlings were assumed negligible.

Net N retranslocation ratio (*NRR*) was computed as:

$$NRR = (A - B) \times A^{-1} \times 100\% \tag{2}$$

Net amount of N derived from soil (*ANDFS*) was computed as:

$$ANDFS = C - NRA \quad (3)$$

Where, C is the total N content in new tissues (needles + new stem + white fine roots in 0–2 mm diameter) on July 2 or September 21.

Net ratio of N derived from soil (*RNDFS*) was computed as:

$$RNDFS = ANDFS \times D^{-1} \times 100\% \quad (4)$$

Where, D is the total N content in initial soil and fertilizer.

N utilization efficiency (U) was computed as:

$$U = Bio \times D^{-1} \times 100\% \quad (5)$$

Where, *Bio* was the dry biomass of the whole seedling or combined stem and root on July 2 or September 21.

Fertilizer efficiency (*FN*) was computed as:

$$FN = Bio \times E^{-1} \quad (6)$$

Where, E was total N content in fertilizer.

Seedling biomass, N concentration, and N content (biomass multiplied by N concentration) were averaged for the 10 seedlings per plot on each sampling date. These values, as well as *NRR*, *ANDFS*, *RNDFS*, *U*, and *FN*, were analyzed using analysis of variance (ANOVA) based on the General Linear Model (GLM) procedure of SAS (9.0) (SAS Institute Inc., NC, USA). For analyses of variance, the effects of treatment (F) and tissue (T) (needles, old stems, new stems, and coarse, medium and fine roots) were considered fixed. When treatment effects were significant, means were ranked according to Tukey's studentized range test at $\alpha = 0.05$. When the effects of T or F were significant, a one-way analysis of variance (ANOVA) was employed to compare means among tissue fractions or treatments, respectively. For biomass and N status data, the interaction of T and F was significant and a two-way ANOVA was employed. Instantaneous vector diagnosis (Salifu and Timmer 2001) was employed to facilitate interpretation of the effects of fertilization treatments on whole seedlings or on combined stem and root growth. Nutritional interpretations of directional vector changes in biomass, N content, and N concentration on September 21 follow the conventions of Salifu and Timmer (2001).

Results

Biomass, N concentration and N content

Fertilization significantly affected biomass and N content on July 2 (Table 1). Seedlings fertilized with 24 $kg \cdot ha^{-1}$ N had the greatest biomass (0.58±0.14 g and highest N content (11.89±4.08 mg), followed by seedlings fertilized with 59 $kg \cdot ha^{-1}$ N (0.50±0.12 g biomass and 10.48±4.08 mg N content). Seedlings fertilized with 117 $kg \cdot ha^{-1}$ N and the control had the least biomass (0.44±0.12 g) and lowest N content (8.93±3.28 mg). On September 21, seedlings fertilized with 24 $kg \cdot ha^{-1}$ N had the greatest biomass (1.61±0.26 g), followed by seedlings in the control treatment (1.50±0.20 g). Fertilization rate did not affect the ratio (R/S) of root and shoot biomass on either July 2 ($p = 0.4964$) or September 21 ($p = 0.3836$). R/S ranged between 0.22 and 0.26 in July, and 0.32 to 0.48 in September.

Table 1. ANOVA for seedling tissue (*T*) and fertilization treatment (*F*), and their interaction on biomass, and N concentration, and N content on July 2 and September 21, 2009.

Date	Source of variation	*df**	Biomass		N concentration		N content	
			MS&	*Pr>F*	MS	*Pr>F*	MS	*Pr>F*
Jul. 2	*T*	5	4.76	<0.0001	934.76	<0.0001	3975.35	<0.0001
	F	4	0.05	<0.0001	12.85	0.1728	26.47	0.0003
	T×F	20	0.01	<0.0001	8.67	0.3598	13.24	0.0003
	Error	60	0.004	-	7.77	-	4.23	-
Sep. 21	*T*	5	8.67	<0.0001	279.12	<0.0001	1276.42	<0.0001
	F	4	1.05	0.0008	0.57	0.5713	275.90	0.0572
	T×F	20	0.22	0.3367	0.18	0.1784	137.44	0.2293
	Error	60	0.19	-	36.93	-	107.55	-

* *df*, degree of freedom. & MS, mean square.

On both sampling dates, biomass, N concentration, and N content differed significantly between tissues (Table 1). Needles had the greatest biomass and N content (Table 2). Compared with new stems, old stems had substantially more biomass and higher N content and N concentration. On July 2, the coarse roots (>5 mm in diameter) had most biomass, while the fine roots (0–2 mm in diameter) had the highest N concentration and content. On September 21, fine roots had more biomass than coarse roots (2–5 mm diameter). Neither N concentration nor N content differed among three root diameter classes in September (Table 2).

The interaction of the effects of fertilization treatment and tis-

sue were significant for biomass and N content on July 2 only (Table 1). Needles had greatest biomass and highest N content in treatments fertilized with 24 kg·ha^{-1} and 59 kg·ha^{-1} N (Fig.1A, C). Old (2008) stem biomass was greatest in control (0) and 24 kg·ha^{-1} N samples (Fig. 1B), while N content in old stems was highest at 24 kg·ha^{-1} N (Fig. 1D).

Table 2. Biomass and N concentration and content in seedling needles, new stems, old stems, and roots of diameters >5, 2-5, and 0-2mm on July 2nd and September 21st, 2010. (mean ± SE)

Tissue	Jul. 2			Sep. 21		
	Biomass (g)	N concentration (mg·g^{-1})	N content (mg)	Biomass (g)	N concentration (mg·g^{-1})	N content (mg)
Needles	1.60±0.05a*	26.88±0.58a	42.89±1.54a	2.25±0.11a	13.52±0.63ab	30.46±2.08a
New stem	0.25±0.01b	12.54±1.10c	6.63±0.59b	1.41±0.13b	5.72±0.41c	8.08±0.91c
Old stem	0.55±0.02c	18.31±0.96b	4.68±0.39bc	2.18±0.20a	9.10±2.01bc	20.89±5.49ab
Root (>5)	0.05±0.01d	6.85±0.53d	0.32±0.06d	0.73±0.07cd	18.03±2.59a	13.01±2.26bc
Root (2-5)	0.25±0.01c	5.41±0.32d	1.33±0.06d	0.36±0.03d	14.51±1.47ab	5.14±0.67c
Root (0-2)	0.27±0.01c	14.45±0.65c	4.09±0.31c	1.19±0.15bc	11.52±1.55abc	13.32±2.86bc

* Different letters in the same column indicate statistically significant differences according to Tukey's studentized range test at the 0.05 level.

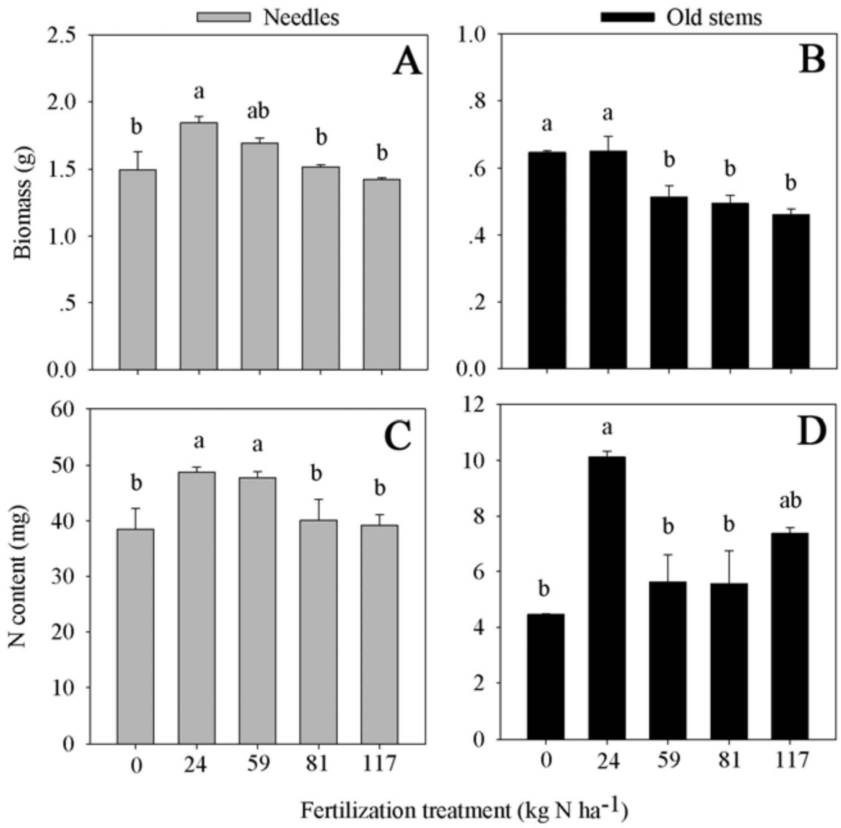

Fig. 1 Biomass (top) and N content (bottom) in needles (left) and old stems (right) of seedlings in different fertilization treatments on July 2, 2009. Different letters above the bars indicate statistical differences according to Tukey's studentized range test at $\alpha = 0.05$.

N retranslocation

Fertilization significantly affected N retranslocation on July 2, but not September 21 (Table 3). On July 2, net N retranslocation amount (*NRA*) and ratio (*NRR*) were lowest in the 24 kg·ha^{-1} N treatment, and the amount and ratio of N derived from soil (*ANDFS* and *RNDFS*, respectively) were highest in this treatment (Table 3). On September 21, *NRA* and *NRR* values were negative, and *RNDFS* values were greater than 100%, indicating N gain rather than loss from the year-old tissues (Table 3).

N utilization

N utilization efficiencies of both CSR and whole seedlings were lowest at fertilization rates of 59 kg· ha^{-1} N and 117 kg· ha^{-1} N

(Fig. 2A, B). Among four fertilization treatments, fertilizer efficiencies for both CSR and whole seedlings were highest in the 24 kg·ha^{-1} N treatment (Fig.2 C,D).

Table 3. Net N retranslocation amount (*NRA*) and ratio (*NRR*) and amount of N derived from soil (*ANDFS*) and ratio of N derived from soil (*RNDFS*) in five treatments on sampling dates of July 2 and September 21, 2009. (mean ± SE)

Investigating Date	Fertilizer treatment (kg·N·ha^{-1})	*NRA* (mg·seedling^{-1})	*NRR* (%)	*ANDFS* (mg·seedling^{-1})	*RNDFS* (%)
Jul. 2	0	8.38±0.09a[a]	57.73±0.60a	39.07±4.80b	81.99±1.72b
	24	2.64±0.34b	18.21±2.32b	56.84±1.37a	95.58±0.46a
	59	7.29±1.07a	50.26±7.38a	48.44±2.63ab	86.80±2.24b
	81	7.38±1.31a	50.86±9.03a	41.47±2.54b	85.15±1.61b
	117	5.43±0.66ab	37.41±4.58ab	41.36±2.25b	88.39±1.31ab
	Pr>F	0.0046		0.0093	0.0014
Sep. 21	0	-81.50±18.71a	-561.70±128.95a	170.90±43.44a	194.14±8.65a
	24	-86.59±24.51a	-596.77±168.89a	161.94±39.36a	212.41±15.20a
	59	-47.54±13.70a	-327.66±94.39a	100.92±20.01a	185.49±17.35a
	81	-50.51±11.25a	-348.10±77.52a	118.67±22.25a	172.38±5.49a
	117	-33.27±1.83a	-229.27±12.59a	70.33±2.96a	194.00±17.76a
	Pr>F	0.1558		0.1646	0.4008

[a] Different letters in the same column indicate statistically differences according to Tukey's studentized range test at α = 0.05.

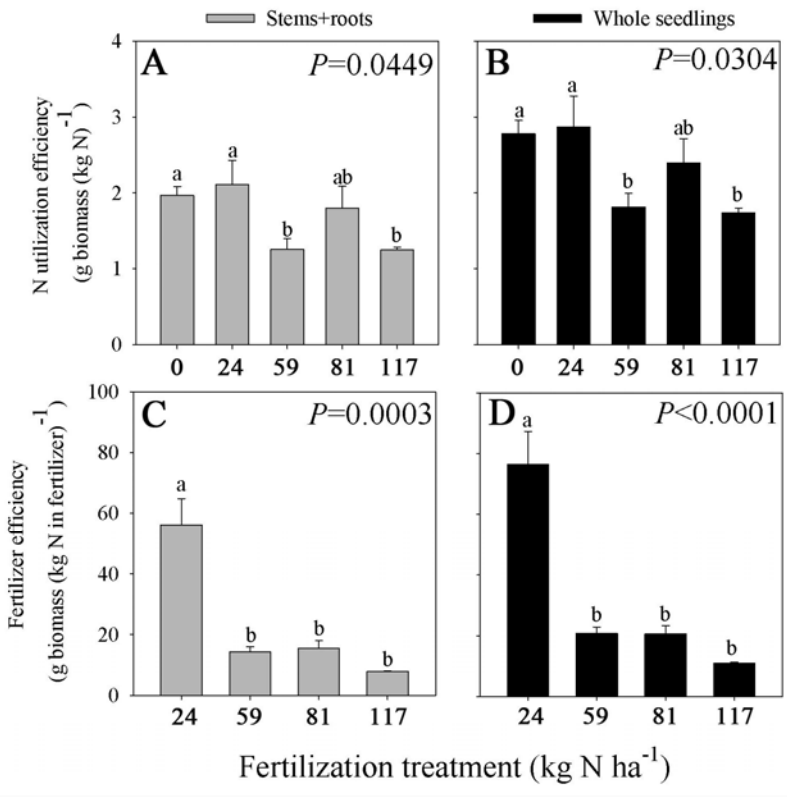

Fig. 2 N utilization efficiency (top) (biomass accumulation (g) /sum of available N content in initial soil and N fertilization amount (kg)) and fertilizer efficiency (bottom) (biomass accumulation (g) /total N content in fertilizer (kg)) in CSR (left) and whole seedlings (right) in different fertilization treatments on September 21, 2009. Different letters above the bars indicate statistical differences according to Tukey's studentized range test at *α* = 0.05.

Vector analysis

On September 21 fertilization induced an N excess in both needles and CSR relative to the controls (Fig.3). Comparing the treatments with the control, the 81kg·ha^{-1} and 117 kg·ha^{-1} N resulted in N depletion in needles (Fig. 3A) and CSR (Fig. 3B). Compared with the 24 kg·ha^{-1} N, all higher fertilization rates

induced N excess in both needles (Fig. 3A) and CSR (Fig. 3B).

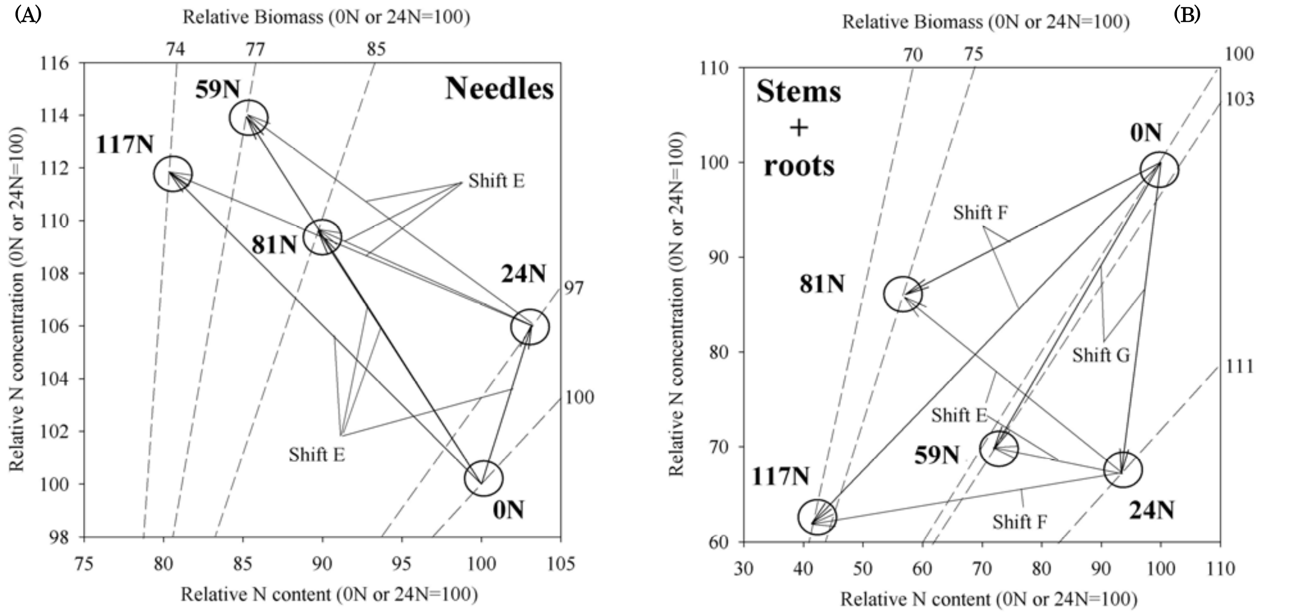

Fig. 3 Vector analysis of N concentration, N content, and biomass of seedlings sampled on September 21, 2009 in four fertilization treatments (24, 59, 81, and 117 kg·ha^{-1} N) plus an unfertilized control (0N) in needles (A) and CSR (combined stems and roots) (B). Seedlings receiving no fertilizer (0N) or 24 kg·ha^{-1} N (24N) were normalized to 100 compared to all fertilization treatments or higher rate fertilization treatments, respectively. Shift E and F indicate nutrient excess. Shift G indicates nutrient depletion (adapted from Salifu and Timmer 2003).

Discussion

Negative effects on biomass of fertilization treatments at rates higher than 24 kg·ha^{-1} N on both sampling dates may relate to the high initial NO_3^--N content in our soils (277.98 mg·kg^{-1}). Levels of NO_3^--N in the Jianmifeng Nursery are high compared to those of other bare root seedling nurseries, for example 0.9 mg·kg^{-1} (Islam et al. 2009). More biomass in needles of our Changbai larch (Table 2) concurred with carbon allocation pattern in saplings of another *Larix* species (Kagawa et al. 2006). More N allocation to needles than other tissues (Table 2) indicates a high photosynthetic capacity in Changbai larch. More biomass and N accumulation in old, woody stems than in new stems (Table 2) suggested an accumulation of N reserves in CSR as observed in other deciduous tree species (Weinbaum and van Kessel 1998; Millard et al. 1998, 2001; Dyckmans and Flessa 2001; Frak et al. 2002; Silla and Escudero 2003). Higher N concentration in fine roots than coarse roots on July 2 (Table 2) concurred with the results reported by Gordon and Jackson (2000), indicating higher nutrient uptake activity in finer roots. On September 21 no significant differences in N concentration among roots of different diameters (Table 2) may have been due to N retranslocation from fine roots as reported by Nambiar (1987).

Compared to other fertilization treatments on July 2 (Table 3) lower *NRA* and *NRR* with higher *ANDFS* and *RNDFS* of seedlings fertilized with 24 mg·kg^{-1} suggested a clear influence of soil N supply on N retranslocation. This contrasts with the conclusion that retranslocation is driven by the magnitude of plant nutrient reserves and is unaffected by current supply (Nambiar and Fife 1991; Millard and Proe 1993; Hawkins and Henry 1999; Salifu and Timmer 2001, 2003). Our results, however, concur with van den Driessche (1985) who found an adequate current supply of N reduced the amount of N retranslocation. For Changbai larch seedlings in north-eastern China, limited top-dress applications of N in the first growing season may not facilitate adequate N reserves. Despite the discrepancy among studies associated with different plant materials and methods, we conjecture that sufficient current N supply tends to influence N retranslocation in seedlings with inadequate N reserves.

Negative values of N retranslocation on September 21 in our study (Table 3) contrast with the research results of Salifu and Timmer (2001), where N retranslocation diminished within 120 days after transplanting, but still showed positive values. Our results, however, concur with Imo and Timmer (2001), where current fertilization reduced N retranslocation in all sites and resulted in net assimilation of N (negative values of retranslocation) on low-competition and nutrient poor sites. In early July, soil temperature begins to rise in northeast China, and most seedling roots may have just begun to function effectively for soil nutrient uptake. Then, presumably, N retranslocation was complete before mid September due to low N reserves or because of abundant N content in nursery soil. Larch seedlings may require more N assimilation than retranslocation due to their high growth rates.

Greater biomass and N content in needles and old stems in the 24 kg·ha^{-1} N treatment on July 2 (Fig. 1) revealed benefits of this treatment in terms of N accumulation during the growing season. Higher N utilization efficiencies of whole seedlings (Fig. 2A) and CSR (Fig. 2B) and higher fertilizer efficiency (Fig. 2C, D) on September 21 in treatments with 24 kg·ha^{-1} N than in other treatments were associated with more biomass and N accumulation in needles and old stems (Fig. 1).

On September 21, lower biomass and N content in the three highest N fertilization treatments indicated an excess of N compared to the control and 24 kg·ha^{-1} N treatment (Fig. 3). The local operational N application rate of 81 kg·ha^{-1} N combined with the already high initial N content of soils, appeared to exceed the N requirement of Changbai larch seedlings (Fig. 3). High nitrate content in local soils may be due to enhanced nitrification in response to decades of continuous N application using ammonium phosphate as fertilizer. Cruz et al. (2009) found low specific denitrification and high specific nitrogenase activities with low N availability in soils as long as 37 years after annual applications of ammonium phosphate. To improve fertilization efficiency of Changbai larch seedlings, it is recommended: (1) to adopt a lower dose of N application than 81 kg·ha^{-1} N, for example, 24 kg·ha^{-1} N; (2) to use other fertilizers instead of ammonium phosphate, for example, controlled-release fertilizer; (3) to add nitrification inhibitor into soils; (4) to fertilize using nutrient loading techniques or performing organic amendment.

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